The University of Chicago

THE EFFECT OF TESTOSTERONE PROPIONATE ON THE URINARY EXCRETION OF ANDROGENS AND ESTROGENS IN EUNUCHOIDISM

A DISSERTATION SUBMITTED TO THE FACULTY
OF THE DIVISION OF THE BIOLOGICAL SCIENCES
IN CANDIDACY FOR THE DEGREE OF DOCTOR
OF PHILOSOPHY

DEPARTMENT OF BIOCHEMISTRY

1939

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WALTER HUGH HOSKINS

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THE EFFECT OF TESTOSTERONE PROPIONATE ON THE URINARY EXCRETION OF ANDROGENS AND ESTROGENS IN EUNUCHOIDISM[1,2,3]

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WHILE THE normal pattern of androgen and estrogen excretion by the human kidney is gradually becoming clearer, the sources of the various constituents are still in part obscure and the amounts of testis secretion required to produce normal amounts of biologically active excretion products are not well known.

A few studies on recovery of injected androgens in man are available. Kochakian (1) administered urinary extracts to one normal and to one castrate man. In the normal, injections of 11 to 17 capon units (his own unit) on two occasions caused no apparent increase in androgen excretion in the urine. In the castrate the injection of 30 to 40 capon units per day for 10 days was followed by a recovery of 3% of the injected activity from the urine passed during this period. Callow (2) reports the assays of Greenwood, using the capon's comb, on the urinary excretion of androgens by 17 men with prostatic hypertrophy, receiving from 10 to 100 mg. of testosterone propionate weekly. Significant increases occurred in only 2 patients, who received 50 and 100 mg. respectively, the values in these rising from 15 to about 50 I.U. during treatment. In two eunuchs one receiving 70 and one 150 mg. of testosterone propionate, he recovered 'some 16 per cent of the administered activity' in the first and a 'still lower percentage' in the second. Callow writes discouragingly that:

> In general, the destruction and inactivation of male hormone in the body after absorption is so intense and the yield of administered hormone recovered in the urine is so small that the present method of urinary androgen assay is unlikely to give results which have any significance in respect to the career of male hormone in the body.

McCullagh, Rumsey, and Cuyler have, however, briefly reported conclusions of a different tenor (3) and through the kindness of Dr. McCullagh we have been able to read the complete manuscript. They report the results of 102 assays for urinary androgens in 14 cases of hypogonadism. Doses of testosterone propionate ranging from 5 to 100 mg. were given in single injections and the androgen excretion followed for 1 to 6 days. They summarize their findings on hypogonads as follows:

> It appears from these observations that in cases of frank hypogonadism, 5 to 10 mg. doses may be expected to cause a rise in urinary androgens toward but not within normal range. Such a rise is maintained for little more than one day. Larger doses raise these levels further and maintain them within normal range for longer periods depending upon the size of the dose given. If the total quantity of

[1] These investigations were supported in part by grants from the Rockefeller Foundation.
[2] We are indebted to Drs. Gregory Stragnell, Erwin Schwenk, and Max Gilbert of the Schering Corporation for the testosterone propionate.
[3] The spectrographic examination was carried out by Drs. T. R. Hogness and A. E. Sidwell of the Department of Chemistry, The University of Chicago.

androgen excreted is calculated in terms of androsterone, it appears that in these cases amounts varying between approximately 20% and 70% of the injected quantities can be accounted for.

Dorfman and Hamilton (4) have recently made numerous spot assays before, during, and after the treatment of a 35-year-old man with one intra-abdominal testis with 20 mg. of testosterone propionate per day for 30 days. From an average initial value (chick-comb assay) of 13 I.U. per day the androgens rose and varied between 47 and 97 I.U. per day during treatment and had fallen to the control level by the 30th day after stopping treatment. These authors estimate 6.2% recovery if the excreted androgen be testosterone, 41% if it be androsterone, 124% if it be dehydroandrosterone, 62.4% if it be divided equally between them, taking the activity of 0.3 mg. of dehydroandrosterone as equalling that of 0.1 mg. of androsterone. These authors also report enormous increases (to 500 I.U. per day) in urinary androgens after daily oral administration of 60 mg. of testosterone propionate to this same patient and to a eunuchoid without clinical response to treatment, and concluded therefore that such material is absorbed and excreted so rapidly that no influence in the organism is possible. This conclusion is apparently based on the subjective experiences of their patients and accordingly requires confirmation.

Kochakian (1) injected castrate dogs with 125 to 400 capon units (Kochakian's unit) of urinary extract, during periods of 1 to 3 days, and recovered from 0 to 5% of the injected activity in the urine in 4 days. Ninety-six to 126 capon units of androstenedione (which has the same activity as androsterone) per day, given to each of 2 castrate dogs for 3 days, yielded 2 and 4% urinary recovery in 7 days. Injection of 3 and 5 capon units per day for 19 to 22 days into 2 rabbits with testicular tumors resulted in the recovery of 3 to 6% of the activity in the urine during this time.

The possibility of the conversion of androgens into estrogenic substances in the body has been considered by many investigators. Zondek (5) discovered that the stallion excretes tremendous quantities of estrogens in the urine and suggested that this indicates the metabolic pathway for the testis hormone in this animal. The somewhat anomalous properties of certain androgens in producing such reactions as breast and uterine enlargement and estrus have raised the same questions; Nelson and Merckel (6), for example, state, "the marked tendency of cisandrostenediol and dehydroandrosterone to produce periods of estrus in normal female rats may be due to their conversion by the ovary to an estrogenic substance."

Steinach, Kun, and Peczenik (7a) reported an increase in urinary estrogens in normal and castrate male rats after the administration of urinary extracts and of androsterone and Steinach and Kun (7b) later confirmed this in men. They injected men of various ages with 50 mg. doses of testosterone propionate 3 times weekly until 1000 mg. had been given. Only the morning sample of urine was collected. This urine was concentrated to one-fifth volume under vacuum and extracted by shaking twice with hot benzene, no separation of androgens and phenolic estrogens being accomplished. Pre-treatment values of urinary estrogens of 0 to 36 R.U. per liter rose during androgen administration to 67 to 110 R.U. during the first week and reached their highest value of 1200 R.U. per liter of morning urine after 7 weeks of treatment, returning to normal in 3 weeks. These high values are especially remarkable in view of the almost casual extraction conducted by shaking the urine twice with benzene and the absence of hydrolyzing procedures usually necessary to secure maximum yields of estrogens from urine. Callow (2) and Webster (8) have indicated in papers not directly concerned with this problem that they have data confirming Steinach and Kun, in eunuchs and in adolescent hypogonad males, respectively; but their results are not as yet available in detail. Kochakian (9) could establish no

such effects from urinary androgens, androstenedione, and several testosterone compounds given in adequate doses to 1 normal and 4 castrate dogs.

If such conversion of androgens into estrogens in man is a physiological phenomenon, the long puzzling question of the source of urinary estrogens in adult men may be at least in part solved. These estrogens pass into the phenolic fraction (10) when this is separated by solubility in sodium hydroxide from the androgen fraction of urine extracts and hence cannot be any known androgen. Dorfman, Gallagher, and Koch (11) observed that the action of the estrogenic material from male urine on the rat uterus was identical with that of theelin (estrone) and unlike that of theelol (estriol). Dingemanse, Laqueur, and Mühlbock (12) have recently crystallized estrone from male urine but hold that it is only partly responsible for the estrogenic activity of such urine, some of the activity residing in a non-ketonic fraction. Our own data on man are seen to be ambiguous in providing the sort of parallel between androgen and estrogen excretion that one would expect if a conversion of one into the other were a consistent occurrence in the body. The phenolic estrogens of normal male urine, ranging from the equivalents of some 2 to 29 γ of estrone per day, did not vary in parallel with the androgens, during the 6-week observations on 4 normal young men examined in this laboratory (10). The seven eunuchoids, however, who averaged a third of the normal amount of androgens, excreted an average of only 1.8 γ of estrone equivalents per day as compared to the average normal of 10 (13).

As the influence of administered testosterone on the excretion of urinary androgens and estrogens is still uncertain, opportunity was taken in the course of certain metabolic studies (14) on hypogonad men to examine these points. The daily intramuscular injection of 25 mg. of testosterone propionate used was sufficient to cause enlargement of the penis and of the prostate, to increase the frequency of erections and in protracted experiments to increase the sexual hair and deepen the voice in such patients, in our experience (15) and in that of others (8, 16, 17). In the metabolic observations made in parallel there occurred a striking retention of nitrogen, sodium, and usually of chloride, with gains in body weight due largely to water held in association with the nitrogen and salts, so that comparisons may be made between the excretion of the biologically active substances and the effects of the hormone in the organism.

METHODS

Complete urine collections were made on each patient including a control period of 7 to 13 days before the injections and a period of 8 to 12 days after treatment ceased. The urines in *F. R.* and *M. D.* were collected without added preservative, in *N. D.* and *H. S.*, 5 cc. of concentrated sulfuric acid was added to each gallon container. In all 4 cases the urine was hydrolyzed and extracted as soon as possible, always within 3 weeks after the start of the collection period, and usually sooner. The urine of *H. S.* was kept in the refrigerator until hydrolyzed, the others largely in the refrigerator, but also a few days at room temperature. Control experiments have demonstrated that no loss in activity is observed after 3 weeks in the refrigerator. We agree with Callow (2) in observing a considerable loss of activity after prolonged standing at room temperature, amounting in our experiments to 40% after 3 months.

After the removal of small portions for the chemical analyses (15) the successive 24-hour samples were combined into 5 to 13-day lots, one-tenth the volume of concentrated commercial hydrochloric acid added, and the acidified urine boiled for 15 minutes over a free flame thus hydrolyzing the conjugated forms in which the androgens normally appear (18) and avoiding the losses consequent upon prolonged boiling

(2, 19, 26). The urine was then extracted with benzene in a continuous extractor (10) in which each volume of urine comes in intimate contact with 12 volumes of benzene. Two and one-half liters of urine can be completely extracted in 4 hours. The benzene-soluble material was shaken with sodium bicarbonate to remove inert substances and then with sodium hydroxide to separate the phenolic estrogens. Non-phenolic androgens, some of which may have estrogenic properties are thus excluded from the alkali-soluble fraction. The neutral or androgenic fraction was dissolved in olive oil and assayed by the capon method of Gallagher and Koch (10). Seven capons were used on each sample and a standard preparation was always run in parallel.

The estrogenic fractions were assayed on spayed rats by an unpublished modification of the Gustavson-D'Amour method (20). Each preparation is injected into 20 spayed rats, given in one dose in 0.25 cc. of sesame oil, each rat having been primed 48 hours previously with 1 γ of estrone. Vaginal smears are taken after 48 and 52 hours. The number of rats showing full estrous smears at either reading is determined for each preparation. Two groups of 20 rats each, receiving 0.5 and 0.75 γ of estrone are run as controls in parallel in each assay. A standard curve was established with 100 rats run on doses of 0.0, 0.25, 0.50, 0.75, and 1.00 γ of estrone on 10 successive weeks. Each point on this curve represents 10 assays with 20 animals on each dose level. Between the points for 0.25 and 0.75 γ of estrone, the relation between gamma of estrone injected and per cent of rats in estrus is practically a straight-line function. This relationship is utilized as follows: the per cent of estrus observed on an unknown is multiplied by the value obtained by dividing the per cent of estrus on the standard curve for the 0.5 γ estrone by the per cent of estrus on 0.5 γ of estrone observed in parallel with the unknown. This correction raises or lowers the observed response to the slope of the standard curve and the amount of estrone in each unknown can then be read off. This correction is applied in order to minimize variations in sensitivity observed in the colony from week to week. Reference to a standard curve gives the statistical benefits of large numbers of animals while the use of 2 control solutions with each set of assays gives a constant check on the sensitivity and accuracy of the method. In an unselected series of 42 weekly assays carried out on this basis 37 gave a higher percentage of estrous response in the group receiving the 0.75 γ standard than in the 0.50 γ group. In 3 assays the lower dose gave the higher response. In 2 assays neither standard produced estrus. The rats used for these 2 assays were old and were subsequently discarded. In the 37 assays where the response to the standards was in the proper order, the per cent of rats in estrus produced by the 0.50 γ control solution varied from 0 to 58% with an average of 25%, on the 0.75 γ solution the response varied from 20 to 80% with an average of 46%, or an average difference of 21% between the two standards. The limit of accuracy of the method is probably 0.1 γ of estrone within the range discussed and the estrogen values reported in this paper are probably accurate within 30%.

DISCUSSION

The androgen and estrogen excretion for each patient, expressed in I.U. of androgens (1 I.U. equals the activity of 0.1 mg. of androsterone) and gamma equivalents of estrone are shown in figures 1, 2, 3, and 4, together with the data on urinary nitrogen and body weight previously described (14). In all instances the values for both androgens and estrogens rose from the subnormal base-line levels immediately after starting testosterone propionate, reaching their highest points during the second week in 2 individuals and

then fell precipitously when treatment was stopped. In 3 of 4 patients, the estrogens declined less rapidly than did the androgens during the offset periods.

The biological effects of testosterone propionate, as exemplified here by the retention of nitrogen and increased body weight become manifest during the period of increased androgen and estrogen excretion and decline in parallel with the declining hormone excretion during recovery. While in 3 individuals (*M. D.*, *N. D.*, and *F. R.*) there was no precise correspondence between the degree of nitrogen and water retention, on the one hand, and

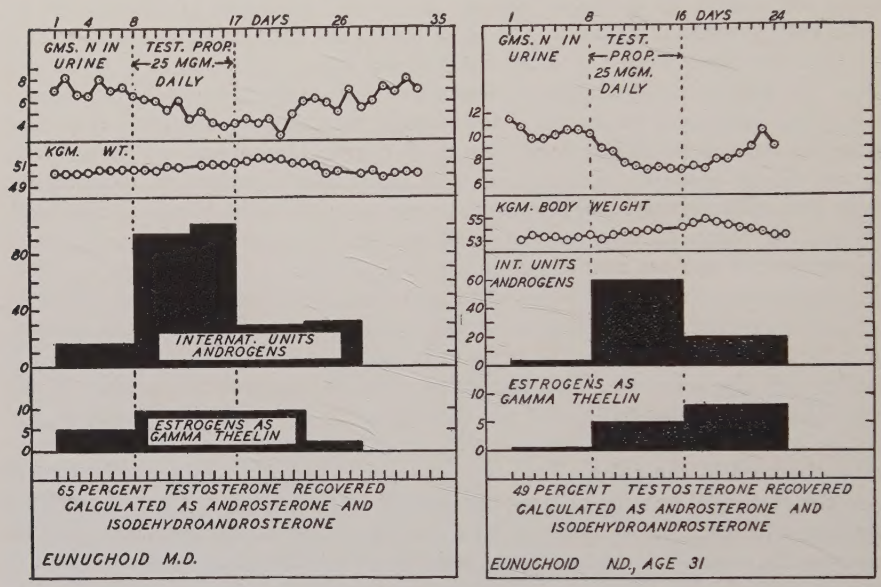

Fig. 1 and Fig. 2

the amounts of androgens and estrogens excreted on the other hand, *H. S.* showed differences from these 3 that are of particular interest. His hypogonadism was due to the injury of the pituitary body by a craniopharyngioma, confirmed at autopsy, while in the others the etiology was unknown, with certainly no evidences of gross pituitary damage. The androgen and estrogen excretion curves in *H. S.* differ in that the rise in androgens is much smaller and that there is an immediate drop to pre-treatment levels after stopping the injections in contrast to the more gradual return otherwise found. The smaller response to testosterone administration evident in the curve for androgen excretion is associated with a slighter degree of nitrogen retention (1.16 gm. N per day as compared to 2.12, 2.39, and 4.51) and the more rapid decline in androgen excretion during recovery is associated with a much sharper rise of nitrogen excretion. To what extent these peculiarities of *H. S.* are due to his hypopituitary state, which, of course, would have affected other tissues as well as the gonads and to what extent they are chance variations, only further work can determine.

In all 4 cases, testosterone propionate increased androgen and estrogen excretion sufficiently to bring the values within normal limits, which for men, in the experience of this laboratory, is for androgens, 22 to 132 I.U., on the basis of the 15-minute hydrolysis, giving an average of 67 I.U.; for the estrogens, the equivalents of 2 to 26 γ of estrone with an average of 10 (10). This suggests that the normal estrogens of male urine may well have their origin in large part in the metabolism of the testis hormone; thus concurring in Zondek's conception of the source of the estrogens in stallion's urine. The parallel fall in estrogens and androgens in the urine of eunuchoids

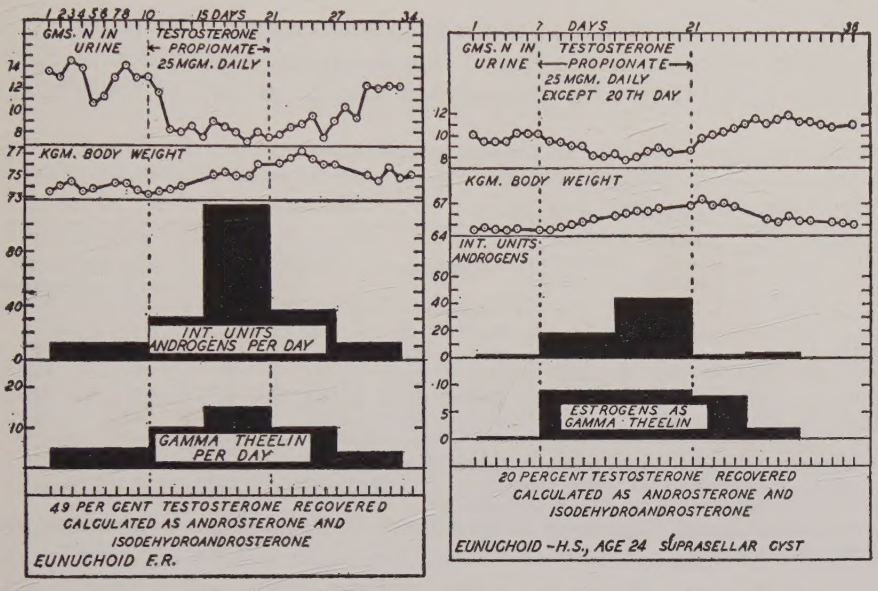

Fig. 3 and Fig. 4

as evidenced by the control values in these experiments, and in previous studies (13) is in agreement with this view and the apparently conflicting observation that the androgen-estrogen ratio in the urine of normal men (10) varied widely may possibly be accounted for by the considerable lag in estrogen excretion as compared to that of the androgens after discontinuing testosterone propionate. Thus physiological lowering of testis secretion might well be reflected later in the estrogens than in the androgens and any precise correlation between the two in any given collection period would accordingly not be expected. The continued excretion, however, of small amounts of estrogens in the urine of castrate men (3 to 4.5 γ of estrone per liter in 2 men [13], 2 to 17 M.U. per liter in 4 men [21]) points to an extragonadal origin for some of this material.

The conversion of testosterone to phenolic estrogens is, however, quantitatively very limited in man. In the case of *M. D.*, for example, the excess estrogens, calculated as estrone, represent 0.127 mg., or only 0.06% of the testosterone given; even when proper allowances are made for the sub-

stantial destruction of estrogens in the body itself the fraction of the testosterone thus transformed is minute. The pure estrogens are, however, so very active that it is quite possible that even these small amounts derived from testis secretion may be of physiological importance and thus account for the occasional breast hypertrophy produced in the eunuchoid by testosterone treatment (13, 17) and for the slight breast swellings of the normal pubescent boy (22). The fact that our values fall considerably short of those of Steinach and Kun (7b), previously mentioned, may be due to differences in extraction, assay, and unitage, or to the fact that their subjects were apparently not hypogonads.

The exact chemical nature of the androgens excreted after testosterone propionate injections is not known. Spectrographic examination of these fractions indicated that the characteristic absorption band of testosterone

TABLE 1. EXTENT OF RECOVERY OF ADMINISTERED TESTOSTERONE PROPIONATE AS URINARY ANDROGENS

Patient	Mg. of testosterone propionate	Equivalent of testosterone[1]	I.U. of excess androgens excreted	Recovery calculated as					
				Androsterone		Dehydroandrosterone		Equal wts. of androsterone and dehydroandrosterone	
				mg.[2]	%	mg.[3]	%	mg.[4]	%
M. D.	250	210	955	95.5	45.5	239	114	136	65
N. D.	225	189	649	64.9	34.3	162	86	93	49
F. R.	300	252	858	85.8	34.1	214	85	123	49
H. S.	350	296	406	40.6	13.7	101	34	58	20

[1] Equivalent weight of testosterone = weight of propionate × 0.84.
[2] 1 I.U. androgen = 0.1 mg. androsterone.
[3] 1 I.U. androgen = 0.25 mg. dehydroandrosterone.
[4] 7 I.U. androgen = 1.0 mg. urinary androgens if made up of equal weights of androsterone and dehydroandrosterone.

itself was absent. Very slight estrogenic activity was found in the non-phenolic or androgen fraction, and no androgenic activity was present in the phenolic fraction. These tests permit us to rule out the possibilities of the excretion of non-phenolic estrogens or of phenolic androgens following testosterone treatment. Since the biological activity of normal urine resides largely in the androsterone and dehydroandrosterone present, recovery calculations are presented in terms of each alone and in terms of equal amounts by weight of the two (table 1). This last is the usual situation in normal male urine according to the evidence available to date (23, 24, 25, 26). One hundred gamma of androsterone is equal in comb-growth production to 250 γ of dehydroandrosterone, according to the assays of Tschopp and Ruzicka, cited by Koch (27), and a number of determinations in this laboratory. Calculated as androsterone, from 13.7% (*H. S.*) to 45.5% (*M. D.*) of the injected testosterone was recovered during the experiment; calculated as dehydroandrosterone, from 34.3% to 114%, calculated as equal amounts by weight of the two, from 20% to 65%. It is thus seen that substantial fractions of the injected testosterone are recoverable from the urine of the eunuchoid, the only low values being in the hypopituitary *H. S.* Our average for the 3

hypogonads of unknown etiology of 54% (equal weights of androsterone and dehydroandrosterone present) is approximately the same as that of Dorfman and Hamilton (4), 62.4%, in a somewhat different experiment, and of McCullagh et al. (3), 20 to 70%, calculated as androsterone, both in hypogonads. A recalculation of Callow's data in a eunuch (2), if we understand them correctly, gives a recovery value of 63% on the basis used here, as compared with the '16% of administered activity,' as he expresses it, and there is thus no substantial disagreement between his work and ours.

It is of interest to calculate the amount of testosterone which must be secreted and metabolized during a 24-hour period to produce the average normal urinary androgen excretion. Such a calculation involves the assumption that the recovery value of 54% (average of 65, 49, and 49%, omitting the hypopituitary *H. S.*) as obtained in hypogonads after testosterone treatment is equal to the recovery from testosterone metabolism in the normal body, and furthermore that the testosterone propionate is or behaves in this respect as the true testis hormone. Taking, however, the average normal daily excretions as 67 I.U., and calculating on the basis that half the weight of the androgens is contributed by each substance, androsterone and dehydroandrosterone, the combined activity equivalents of which represent 7 I.U. per mg., we have a daily urinary loss of 9.6 mg. of urinary androgens. Since, according to our recovery figures this is only 54% of the amount of testosterone metabolized in the body during a 24-hour period the total testosterone used becomes 17.8 mg. per day. This figure must be reduced to the extent that urinary androgens in man have a non-gonadal source. Castrates certainly excrete some androgenic material and Callow (2) has observed as much as 30 to 39 I.U. per day. The proper average value for this allowance must await more extensive studies. The daily dose of testosterone required to replace the activity of the normal testis in man is not known; but from 7.0 to 25 mg. per day of the propionate will develop the secondary sex characters of the hypogonad and cause enlargement of the penis and prostate (8, 14, 15, 16, 17).

SUMMARY

The intramuscular injection of 25 mg. of testosterone propionate per day for periods of 9 to 14 days to 4 eunuchoids brought about rises in urinary androgens from basal levels of 2 to 16 I.U. per day to 44 to 115 I.U. per day and of urinary estrogens from the equivalents of 0.3 to 5 γ of estrone per day to that of 5 to 15 γ of estrone per day. This constitutes a restoration to normal values for both groups of substances. Both androgen and estrogen values fell toward pre-treatment levels when injections stopped. The changes in androgen and estrogen excretion paralleled the physiological effects of testosterone propionate in the organism.

In 3 patients in whom the cause of the hypogonadism was unknown the extent of recovery of administered testosterone was 65, 49, and 49%, on the assumption that half of the excreted material by weight is androsterone and half dehydroandrosterone. In a patient whose pituitary was damaged by a suprasellar cyst, the recovery was 20%.

The rise in urinary estrogens to normal values following androgen administration suggests that the urinary estrogens of normal male urine may be derived in part from the metabolism of the testis hormone. However, the increase in estrogen excretion represents only 0.06% of the androgen injected.

REFERENCES

1. Kochakian, C. D.: *Endocrinology* 21: 60. 1937.
2. Callow, R. K.: *Proc. Roy. Soc. Med., London*, 31: 841. 1938.
3. McCullagh, E. P., J. M. Rumsey and W. K. Cuyler: *Proc. Cen. Soc. Clin. Res.* 11: 18. 1938; personal communication.
4. Durfman, R. I., and J. B. Hamilton: *J. Clin. Investigation* 18: 67. 1939.
5. Zondek, B.: *Nature* 133: 209, 494. 1934.
6. Nelson, W. O., and C. Merckel: *Proc. Soc. Exper. Biol. & Med.* 36: 825. 1937.
7. a. Steinach, E., H. Kun and O. Peczenik: *Wien. klin. Wchenschr.* 29: 899. 1936.
   b. Steinach, E., and H. Kun: *Akad. Anzeiger Wien.* vol. 15. 1937.
8. Webster, B.: *J. Pediat.* 13: 847. 1938.
9. Kochakian, C. D.: *Endocrinology* 23: 463. 1938.
10. Gallagher, T. F., D. H. Peterson, R. I. Dorfman, A. T. Kenyon and F. C. Koch: *J. Clin. Investigation* 16: 695. 1937.
11. Dorfman, R. I., T. F. Gallagher and F. C. Koch: *Endocrinology* 19: 33. 1935.
12. Dingemanse, E., E. Laqüeur and O. Mühlbock: *Nature* 141: 927. 1938.
13. Kenyon, A. T., T. F. Gallagher, D. H. Peterson, R. I. Dorfman and F. C. Koch: *J. Clin. Investigation* 16: 705. 1937.
14. Kenyon, A. T., I. Sandiford, A. H., Bryan, K. Knowlton and F. C. Koch: *Endocrinology* 23: 135. 1938.
15. Kenyon, A. T.: *Endocrinology* 23: 121. 1938.
16. Vest, S. A., Jr., and J. E. Howard: *J. Urology* 40: 1. 1938.
17. Hamilton, J. B.: *Endocrinology* 21: 649. 1937.
18. Peterson, D. H., W. H. Hoskins, J. R. Coffman and F. C. Koch: *Proc. Am. Soc. Biol. Chem.* 32: xviii. 1938.
19. Peterson, D. H., T. F. Gallagher and F. C. Koch: *J. Biol. Chem.* 119: 185. 1938.
20. D'Amour, F. E., and R. G. Gustavson: *J. Pharmacol. & Exper. Therap.* 40: 473. 1930.
21. Eng, H.: *Wien. klin. Wchnschr.* 15: 349. 1936.
22. Jung, F. T., and A. L. Shafton: *Proc. Soc. Exper. Biol. & Med.* 33: 455. 1935.
23. Butenandt, A., H. Dannenbaum, G. Hanisch and H. Kudszus: *Ztschr. f. physiol. Chem.* 237: 57. 1935.
24. Schoeller, W., A. Serini and M. Gehrke: *Naturwiss.* 23: 337. 1935.
25. David, K.: *Acta brev. Neerl.* 5: 31. 1935.
26. Dingemanse, E., and E. Laqueur: *Biochem. J.* 32: 651. 1938.
27. Koch, F. C.: *Physiol. Rev.* 17: 153. 1937.